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Subcellular location of the enzymes of purine breakdown in the yeast *Candida famata* grown on uric acid

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1. SUMMARY

The subcellular location of the enzymes of purine breakdown in the yeast *Candida famata*, which grows on uric acid as sole carbon and nitrogen source, has been examined by subcellular fractionation methods. Uricase was confirmed as being peroxisomal, but the other three enzymes, allantoinase, allantoicase and ureidoglycollate lyase were shown to be cytosolic. In addition the peroxisomes harboured catalase and the key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase.

2. INTRODUCTION

The breakdown of purines in yeasts and moulds involves the enzymes uricase (urate oxidase), allantoinase, allantoicase and ureidoglycollate lyase [1,2]. Although these enzymes were shown to be peroxisomal in the livers of marine fish [3], only one report has appeared on the subcellular location of these enzymes in yeasts [4], in which it was shown that in *Candida tropicalis* grown on oleic acid as carbon source in the presence of uric acid as nitrogen source, ureidoglycollate lyase was located in peroxisomes. The yeast *Candida famata* can grow on different purines, including uric acid, which can be used as the combined carbon and nitrogen source for growth [5]. This growth is accompanied by proliferation of microbodies (peroxisomes), which were shown by cytochemical staining to contain uricase [6]. However, the location of the other key enzymes of uric acid breakdown is unknown and is the subject of the present report.

3. MATERIALS AND METHODS

3.1. Growth of the organism

Candida famata CBS 8109 was grown in shake flask cultures in the mineral medium described previously [7]. Cells pregrown on 0.5% (w/v) glu-

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cose were shifted to fresh medium containing 1% (w/v) uric acid as combined carbon and nitrogen source and incubated at 30°C; growth was measured as described [6]. Cells were harvested at $OD_{660} = 1.4$, when the uric acid had just finally been used up.

3.2. Analytical procedures

Cell free extracts were prepared as described [6]. Uricase was assayed as described previously [6] with Tris buffer replaced by 0.1 M borate (pH 8.0); allantoinase and allantoicase were determined by the method of Takada and Noguchi [8] and ureidoglycollate lyase as described by Takada and Tsukiji [4] (prior to the ureidoglycollate lyase assay the cell free extract was incubated for 5 min with 2 mM $MnCl_2$). Catalase and cytochrome *c* oxidase were determined as described by Douma et al. [9]; malate synthase and isocitrate lyase by the method of Dixon and Kornberg [10]. Polypeptides in protein fractions were analysed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) [11] and stained with Coomassie Blue.

Enzyme activities are expressed as units min^{-1} mg $protein^{-1}$. Protein was determined by the method of Bradford [12].

3.3. Preparation of spheroplasts and cell fractionation methods

Spheroplasts were prepared by the method of Douma et al. [9] except that apart from zymolyase, 0.4 mg/ml novozym was added for cell wall lysis. 2 M sorbitol was used as an osmotic stabilizer. Spheroplasts were homogenized in a Potter homogenizer and the lysates subjected to differential centrifugation as described previously [9]. The resulting 30000 × *g* pellet (P_4) and supernatant fluid (S_4) were analysed for enzyme activities. The microbody-enriched P_4-fraction was also applied to sucrose density centrifugation as described hitherto [9].

3.4. Electron microscopy

Whole cells and subcellular fractions were fixed and prepared for electron microscopy as has been described [7].

4. RESULTS AND DISCUSSION

4.1. Enzymes of purine breakdown in crude extracts

Crude extracts of uric acid-grown *C. famata* contained high levels of purine degradatory enzymes (Table 1). In the case of the first three enzymes, these activities are approximately 5–6 times higher than in *C. famata* grown on uric acid as sole nitrogen source [4]. For uricase this is the anticipated result since in yeasts the levels of microbody-borne key enzymes involved in the metabolism of a given growth substrate (e.g. purines, but also D-amino acids or primary amines) are invariably higher in those conditions where this compound is utilized as the combined carbon- and nitrogen source, instead of the nitrogen source alone [13].

4.2. Subcellular distribution of purine-degradatory enzymes

Uric acid-grown cells of *C. famata* contain many microbodies, often present in clusters (Fig. 1A), which may occupy 10–12% of the cytoplasmic volume [5]. The distribution of the enzymes involved in purine metabolism of these cells was studied by conventional subcellular fractionation methods. The distribution of the different enzymes after differential centrifugation of lysed spheroplasts is shown in Table 2A. The data presented on the ratio of enzyme activities present in the 30000 × *g* pellet (P_4) and supernatant (S_4) clearly demonstrate that, apart from uricase, also catalase, the key enzymes of the glyoxylate cycle (isocitrate lyase and malate synthase) and the mitochondrial marker enzyme, cytochrome *c* oxidase, were sedimentable, whereas the other enzymes measured (allantoinase, allantoicase and

Table 1

Enzymes of purine degradation in uric acid-grown *Candida famata*

Enzyme	Specific activity (units/mg protein)
Uricase (EC 1.7.3.3)	1.05
Allantoinase (EC 3.5.2.5)	1.08
Allantoicase (EC 3.5.3.4.)	0.92
Ureidoglycollate lyase (EC 4.3.2.3)	0.074

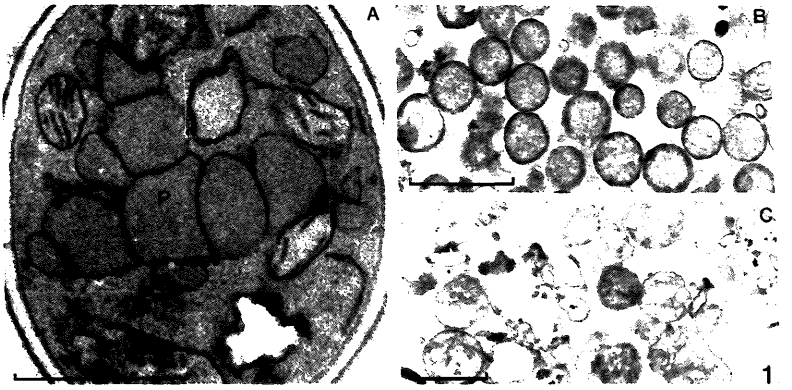

Fig. 1. Electron micrographs. (A) Thin section of a $KMnO_4$-fixed cell showing the typical cluster of peroxisomes (P) in uric acid-grown *C. famata*. (B, C) Electron micrographs of isolated peroxisomes (B) and mitochondria (C) as they were present in the catalase- and cytochrome c-oxidase peak fractions after sucrose gradient centrifugation. The marker represents 0.5 μm.

ureidoglycollate lyase) remained in the supernatant fluid. A further separation of the sedimentable enzymes in the P_4-pellet fraction was obtained after sucrose density centrifugation. A peak of uricase, isocitrate lyase and malate synthase co-sedimented in a narrow band of protein, at approximately 54.5% sucrose, together with catalase. Electron microscopy revealed that these fractions consisted of highly purified peroxisomes (Fig. 1B). The above enzymes were clearly separated from cytochrome *c* oxidase which was localized at approximately 43% sucrose in the mitochondrial fraction (Fig. 1C). Hence, of the enzymes involved in purine breakdown in *C. famata*, only uricase is peroxisome-bound while the other enzymes, allantoinase, allantoicase and ureidoglycollate lyase, are cytosolic in their location. This finding is in agreement for the first two enzymes with the conclusions of Tanaka and Tsukiji [4], but not for ureidoglycollate lyase. These authors were unable to demonstrate activity of this enzyme in crude extracts, due to its marked instability in *C.*

Table 2

Cell fractionation of uric acid-grown *Candida famata*

A. Ratio (P_4/S_4) of enzyme specific activities (units/mg protein) present in the 30000 × g pellet (P_4) and 30000 × g supernatant (S_4) obtained after differential centrifugation of homogenized spheroplasts. B. Enzyme distribution in catalase and cytochrome *c* oxidase peak fractions after discontinuous sucrose gradient centrifugation of the P_4 (30000 × g pellet). Enzyme activities in units/ml.

	Prot	Cat	UOX	MAS	ICL	UGL	ALN	ALC	COX
A. P_4/S_4	2.5	3.4	18.5	22.9	1.9	0.14	0.40	0.39	45.3
B. Fraction 6	–	22.5	1.54	0.42	0.61	0.01	0.02	0.00	0.07
Fraction 16	–	5.73	0.16	0.16	0.09	0.02	0.03	0.00	0.60

tropicalis. Consequently, although activity was found in peroxisomal fractions, there was no way of establishing what proportion this was of the total amount of the enzyme in crude extracts. By contrast, we had no difficulty in demonstrating the activity of ureidoglycollate lyase in crude extracts of *C. famata* and the bulk of that activity was recovered in the cytosolic fraction.

The differences in the sedimentation pattern of the peroxisomal-bound enzymes after differential centrifugation of cell lysates is a common observed feature in yeast [14] and most probably has to be explained as preferential leakage of different enzymes (especially catalase and isocitrate lyase) from the organelles, due to the procedures applied, rather than to a partly cytosolic localization. The peroxisomal nature of catalase–and also uricase–in *C. famata* has furthermore been demonstrated cytochemically [6]. The traces of allantoinase and ureidoglycollate lyase activities found in the organellar peak fractions, after sucrose density centrifugation are too low to attribute to peroxisomal- and/or mitochondrial bound material, but are probably due to a minor contamination of these fractions with small cytosolic vesicles.

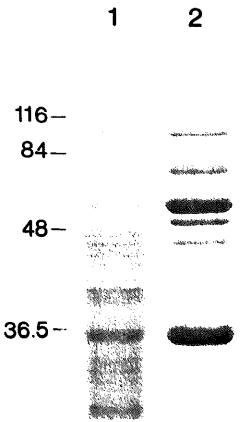

Fig. 2. SDS–PAGE of crude extracts (lane 1) and the peroxisomal peak fraction (fraction 6) after sucrose gradient centrifugation of the 30000 × *g* pellet fraction (P_4) obtained after differential centrifugation of homogenates of uric acid-grown *C. famata*.

The results of SDS–PAGE of the peroxisomal peak fraction indicated that peroxisomes from uric acid-grown *C. famata* contained relatively few matrix proteins (Fig. 2). Based on the subunit molecular mass of the purified enzyme (P.J. Large, unpublished results), the dominant protein band observed at 36 kDa represented uricase; the nature of the second dominant band (a doublet) is not yet clear but may, from the molecular mass, have represented both glyoxylate cycle enzymes [15].

In conclusion, we therefore favour the view that in *C. famata* only uricase is organelle-bound, while the other enzymes of purine degradation are cytosolic.

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